Sodium balance, arterial pressure, and the role of the subfornical organ during chronic changes in dietary salt

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Hendel, Michael D., and John P. Collister. Sodium balance, arterial pressure, and the role of the subfornical organ during chronic changes in dietary salt. Am J Physiol Heart Circ Physiol 289: H426–H431, 2005. First published February 25, 2005; doi:10.1152/ajpheart.01051.2004.—The subfornical organ (SFO), one of the brain circumventricular organs, is known to mediate some of the central effects of angiotensin II related to sodium and water homeostasis. Because angiotensin II levels are altered with changes in chronic dietary salt intake, we reasoned that the actions of angiotensin II at the SFO might be involved in the regulation of arterial pressure during long-term alterations in dietary salt. The present study was designed to test the hypothesis that long-term control of arterial pressure during chronic changes in dietary salt intake requires an intact SFO. Male Sprague-Dawley rats were randomly selected for 4-week periods of normal-salt (1.0% NaCl) diet, electrolytic lesion (SFOx, n = 8) or sham (n = 9) operation of the SFO. After a 1-wk recovery period, rats were instrumented with radio-telemetric blood pressure transducers for continuous 24-h measurement of mean arterial pressure (MAP) and heart rate (HR) and then were placed individually in metabolic cages. After another 1 wk of recovery, the rats were subjected to a 49-day protocol as follows: 1) a 7-day control period (1.0% NaCl diet), 2) 14 days of high-salt (4.0% NaCl) diet, 3) 7 days of normal-salt (1.0% NaCl) diet, 4) 14 days of low-salt (0.1% NaCl) diet, and 5) 7 days of recovery (1.0% NaCl diet). There were no significant differences in MAP or HR between SFOx and sham-operated rats throughout the protocol. These results do not support the hypothesis that the SFO is necessary for regulation of arterial pressure during chronic changes in dietary salt. However, SFOx rats demonstrated significantly less cumulative sodium balance than sham-operated rats on days 2–6 of the high-salt diet period. These data suggest that the SFO is important in the regulation of sodium homeostasis during chronic changes in salt intake.

sympathetic nervous system; neurogenic; osmoreceptor; hypertension; salt sensitive

THE FOREBRAIN CIRCUMVENTRICULAR ORGAN, the subfornical organ (SFO), is a central integration site for the actions of angiotensin II (ANG II) in the regulation of cardiovascular and body fluid homeostasis. The SFO receives hormonal input from peptides such as ANG II, and these interactions may be important in sensing peripheral changes in salt ingestion that directly or indirectly affect long-term regulation of arterial blood pressure. This idea is supported by numerous studies investigating the acute and chronic actions of ANG II at the SFO (13, 19, 28, 37).

SFO neurons become excited with stimulation of ANG II in vivo (41) and in vitro (29), and immunohistochemical mapping studies of the brain reveal that the SFO is densely populated with angiotensin type I receptors (34). Physiological studies of the SFO demonstrate that acute injections of ANG II into the SFO elicit a pronounced dipsogenic effect (28), which is abolished by SFO lesions (37). Additionally, ANG II binding at the SFO has been implicated in initiating salt appetite (37) and release of arginine vasopressin (21).

Furthermore, the SFO is a major site in the forebrain responsible for initiating some of the central pressor effects of ANG II. Acute injections of ANG II into the SFO elicit a pressor response (4, 23) that can be markedly attenuated by preinjections of the ANG II antagonist saralasin (4). Additionally, intravenous ANG II causes a pressor response that can be blocked by lesion of the SFO (19, 27). Many findings suggest that this central pressor effect of exogenously administered ANG II is sympathetically mediated. The central-acting sympatholytic agent clonidine reversed the slow pressor response to chronic ANG II administration in rats (15). Furthermore, direct measurement of sympathetic nerve activity and plasma norepinephrine in rabbits during the chronic phase of ANG II hypertension supports the view that the hypertensive effect is neurogenically mediated (9).

The efferent connections of the SFO support the view that the ANG II-induced pressor effect is via activation of the sympathetic nervous system. Electrophysiological studies demonstrate that SFO neurons project down to the paraventricular nucleus (PVN) (40), and neurons in the SFO provide excitatory input to PVN cells, which further project down to the sympathetic neurons of the intermediolateral cell column (1). Finally, electrolytic lesions of the PVN significantly reduced the SFO-induced pressor response (12).

Numerous studies suggest long-term autonomic responses to changes in dietary salt (2, 14, 24, 38). Moreover, some even propose a direct link between the sympathetic nervous system and salt-dependent hypertension (2, 18, 35). This seems to be the case in several animal models of hypertension, including the spontaneous hypertensive rat (3, 5, 22), the deoxycorticosterone acetate-salt hypertensive rat (10, 39), and the Dahl rat (16, 20). The sympathetic nervous system appears to be directly correlated with hypertension in these animal models, but there is less understanding of the reason for this correlation or its method of operation. Osborn et al. (31) reported that rats with a sympathetic nervous system that is “clamped” by α-adrenergic blockade become salt sensitive and that they are twice as salt sensitive as baroreceptor-denervated rats. Furthermore, in some subjects with salt-sensitive hypertension, the sympathetic nervous system is inappropriately elevated, possibly because of an inability to adequately suppress ANG II levels and, therefore, a failure to suppress the sympathetic activity.
nerve system (2). If long-term changes in dietary salt modulate sympathetic outflow, how are these peripheral signals communicating with the central nervous system? The SFO is one possible site that can sense changes in ANG II levels and modulate sympathetic nerve activity. Therefore, we reasoned that the renin-angiotensin system will respond to chronic changes in dietary sodium and that these changes are sensed at the SFO, which can respond with appropriate neural signals critical to maintaining homeostasis. The present study examines the effect of SFO lesions on long-term control of arterial pressure and sodium and water balance during changes in salt intake.

The present study was designed to test the hypothesis that the SFO is necessary to maintain normotension during chronic changes in salt intake. We hypothesized that animals with a lesion of the SFO will be unable to regulate blood pressure appropriately during chronic changes in salt intake. Rats were subjected to SFO lesion (SFOx) or sham operation (SHAM). They were then fed high- and low-salt diets for 2 wk. During this time, mean arterial blood pressure (MAP) and heart rate (HR) were measured with telemetry in SFOx and SHAM animals for 7 wk.

METHODS

All procedures complied with the Guide for Care and Use of Laboratory Animals (NIH Pub No. 85-22, Revised 1996) and were approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Surgical procedures. Adult male Sprague-Dawley rats (Charles River Laboratories; 275-300 g body wt) were randomly selected for the SFOx or the SHAM group. Rats were preanesthetized with pentobarbital sodium (32.5 mg/kg ip) and atropine (0.2 mg/kg ip). Surgical anesthesia was achieved with a second intramuscular injection containing a cocktail of anesthetic agents: acetylpromazine (0.2 mg/kg), butorphanol tartrate (0.2 mg/kg), and ketamine (25 mg/kg). Rats were then positioned in a stereotaxic apparatus. SFO lesions were completed as previously reported (6). Briefly, a dorsal midline incision was made through the skin of the skull. Bregma and lambda landmarks were exposed, and a 3-mm hole was drilled 1.5 mm posterior to bregma. A Teflon-coated tungsten electrode with 0.008 inch exposed at the tip was passed into the brain at four predetermined coordinates caudal and ventral to bregma, respectively (in mm): −0.8 and −5.2, −1.0 and −5.1, −1.2 and −4.9, and −1.4 and −4.7. At each location, a 1-mA current was passed for 8 s to complete the lesion. The hole in the skull was closed with bone wax, and the skin was closed with 3-0 silk suture. The lesion technique, depicting electrode placement and extent of the lesions, is shown schematically in Fig. 1. Sham operations were identical to lesions, except ventral coordinates were 1.5 mm less and no current was passed. At the end of each surgery, each rat received an antibiotic injection of gentamicin (2.5 mg im) and an injection of butorphanol tartrate (0.075 mg sc) for analgesia.

After 1 wk of recovery, SFOx rats weighed 292 ± 2 g and SHAM rats weighed 294 ± 3 g. There were no significant differences in body weight between SFOx and SHAM rats at any time before the protocol. The animals were then instrumented with radio-telemetric pressure transducers (model TA11PA-C40, Data Sciences International) for the purpose of continuous 24-h sampling of MAP and HR. The rats were anesthetized as described above, and transmitters were implanted as previously described (6). At the end of surgery, each rat received antibiotic and analgesic injections as described above.

Rats were then placed individually in metabolic cages (Nalgene). The telemetry receiver, placed behind the metabolic cages, allowed us to collect urine samples gravimetrically. Rats were then allowed to re feed for a 1-wk recovery period before entering the protocol. Distilled water was provided ad libitum, and the rats were fed a normal-NaCl (1.0%) diet to initiate the control period.

Experimental protocol. SHAM and SFOx rats were fed a normal-salt (1.0% NaCl) diet during the first 7 days of the protocol, which served as the initial control period. The next 14-day period, during which the rats were fed a high-salt (4.0% NaCl) diet, was followed by a 7-day period during which the animals were fed normal-salt (1.0% NaCl) diet. Beginning on day 21, the rats were fed a low-salt (0.1% NaCl) diet for 14 days; then the animals were allowed a 7-day recovery period, which was identical to the control period.

Daily measurements of MAP, HR, food intake, water intake, and urinary sodium were recorded in conscious, unrestrained rats in their home cages. MAP and HR were measured continuously by radio-telemetric pressure transducers at a sampling rate of 500 Hz for 10 s each minute. Gravimetric measurements of 24-h food and water intake and urinary output were obtained. Sodium intake was calculated as the amount of sodium from the diet. Urinary sodium content was measured with an ion-specific electrode (Nova Biomedical). Urinary sodium excretion was calculated as the product of the urine flow rate and urinary sodium concentration.

Confirmation of SFOx. On completion of the experimental protocol, all rats were anesthetized as described above and perfused intracardially with 4% paraformaldehyde. Whole brains were extracted and soaked in 4% paraformaldehyde for 2 days. The brains were then transferred to a 30% sucrose solution for 3 days. Frozen serial sagittal sections (50 μm) were made at the lateral edge of the third ventricle and mounted on slides. The slides were then stained for Nissl substance with cresyl violet stain. Complete SFOx or intact SFO (SHAM rats) was confirmed by light microscopy. Rats were considered lesioned if ≥80% of the SFO was ablated (Fig. 2), with the lesion including the rostroventral efferent fibers of the SFO.

Statistical analysis. Differences between experimental groups were analyzed by a two-way ANOVA using a statistical package (Abacus

Fig. 1. Schematic of subfornical organ (SFO) lesion technique depicting electrode placement (●), coordinates (dashed lines), and extent of electrolytic lesions (dashed circles). 3V, 3rd ventricle.
Comparisons of specific experimental days (within and between groups) were performed by linear contrast analysis. Between-group comparisons of baseline control values were done with an unpaired t-test. \( P < 0.05 \) was considered statistically significant for all tests. Values are means \( \pm \) SE.

**RESULTS**

**Cardiovascular response to chronic changes in salt intake.** Cardiovascular responses to chronic changes in salt intake are shown in Fig. 3. The average MAP for the 7-day control (1.0% NaCl) period was 100 \( \pm \) 2 and 98 \( \pm \) 2 mmHg for SHAM and SFOx rats, respectively. The average MAP for the normal-salt diet phase after the high-salt diet period was 102 \( \pm \) 3.8 and 98 \( \pm \) 2.3 mmHg in SHAM and SFOx rats, respectively. The average MAP for the final normal-salt diet period was 105 \( \pm \) 3.1 and 101 \( \pm \) 1.6 mmHg in SHAM and SFOx rats, respectively. No significant differences in MAP were observed between SHAM and SFOx rats throughout the protocol, even though SHAM rats displayed slightly elevated MAP compared with SFOx rats on most days of the protocol.

The 7-day control average HR was 387 \( \pm \) 6 and 392 \( \pm \) 6 beats/min in SHAM and SFOx rats, respectively. There were no differences in HR between SHAM and SFOx rats throughout the protocol.

**Sodium and water balance.** Daily water balance data are shown in Fig. 4. The 7-day control average water intake was 26 \( \pm \) 2 and 24 \( \pm \) 1 ml/24 h in SHAM and SFOx rats, respectively. Although both groups demonstrated increased water intake during the high-salt diet period, there were no differences in water intake between groups throughout the protocol (Fig. 4, top). The average urine output was 11 \( \pm \) 3 and 9 \( \pm \) 1 ml/24 h in SHAM and SFOx rats, respectively, during the control period. Again, no differences in urine output were detected between groups throughout the changes in dietary salt intake (Fig. 4).

Daily sodium balance data are also shown in Fig. 4. The 7-day control average sodium intake was not different between SHAM and SFOx rats: 4.0 \( \pm \) 0.3 and 4.4 \( \pm \) 0.3 meq/24 h, respectively. Sodium intake tended to be less in SFOx than in SHAM rats throughout the 4% NaCl diet period, although the difference was not significant (Fig. 4). No statistical differences were seen in daily sodium intake between the groups throughout the protocol. In terms of daily sodium excretion, no difference in the 7-day average control values were detected between SHAM and SFOx rats (4.0 \( \pm \) 0.5 and 4.0 \( \pm \) 0.4 meq/24 h, respectively) or during the remainder of the protocol (Fig. 4, bottom). To detect subtle differences in sodium or water homeostasis, in addition to daily measurements of sodium and water balance, cumulative measurements were also calculated for each group. No differences in cumulative water balance were seen between groups throughout the protocol (Fig. 5, top). There was no difference in cumulative sodium balance between SHAM and SFOx rats during the control period; however, a decreased cumulative sodium balance was seen in SFOx rats on initiation of the high-NaCl (4.0%) diet. This was statistically significant on days 2–6 of this dietary
There was no detectable difference in cumulative sodium balance between the groups for the remainder of the protocol.

**DISCUSSION**

The present study was designed to determine whether the SFO is necessary to maintain arterial pressure during chronic changes in salt intake. The results of these experiments do not support this hypothesis (Fig. 3). The arterial pressure remained the same in SFOx and SHAM rats throughout the protocol, including the 14-day high-salt (4.0% NaCl) diet period. There were also no differences between SFOx and SHAM rats during the 14-day low-salt (0.1% NaCl) diet period or any of the normal-salt (1.0% NaCl) diet periods. Additionally, baseline blood pressure was not affected in SFOx rats.

There are several possible explanations for why our hypothesis was not supported in this study. It appears that SFOx rats did not have an impaired ability to maintain normotension when fed high- or low-salt diets. The first explanation is that the SFO may not be important in maintaining cardiovascular homeostasis during chronic changes in dietary salt intake. It is also possible other central systems, such as the organum vasculosum of the lamina terminalis or area postrema, may be compensating for the SFO in this model. Importantly, we also previously reported that the area postrema does not play a role in long-term maintenance of arterial pressure during chronic changes in dietary salt intake (7). Certainly, there is evidence to support the notion that baroreceptors are important to long-term regulation of blood pressure (25, 26). Indeed, some studies suggest that the baroreceptor reflex dysfunction is responsible for some forms of salt-dependent hypertension (32, 33). However, other studies support the idea that the baroreceptors adapt to a sustained stimulus and, therefore, are not involved in chronic blood pressure regulation (8).

Interestingly, despite no differences in arterial pressure, SFOx rats displayed less positive cumulative sodium balance during the initial days of the high-salt diet. Because we did not observe an increase in sodium excretion in SFOx rats during these days, we suspect that this is due to less sodium intake. Although this was not shown to be significant, it could account for the subtle differences in the cumulative sodium balance data. Others have reported a role for the SFO in sodium appetite (36, 42), but this is usually observed after sodium depletion. Even with a subtle difference in food intake, SFOx rats demonstrated an inappropriately high sodium excretion to account for the difference in cumulative sodium balance between SFOx and SHAM rats. This suggests altered neural or hormonal control of renal function in SFOx rats. It has been shown that renal-denervated rats (11, 30) have decreased ability to conserve sodium after sodium depletion. Perhaps SFOx rats have less renal sympathetic nerve activity, and this becomes apparent when they are fed a high-salt diet, inasmuch as they exhibit an inappropriately high sodium excretion. These results are the opposite of our observations in rats with lesions of the area postrema. We previously reported an impaired ability to excrete sodium in area postrema-lesioned rats.
fed a 4.0% NaCl diet (7). The fact that SFOx rats displayed differential cumulative sodium balance compared with SHAM rats while maintaining the same level of normal blood pressure is indeed a critical aspect of the present study. Sodium balance has consistently been linked to arterial pressure regulation (17). The present results demonstrate a dissociation in the correlation between sodium balance and blood pressure regulation.

In summary, we report that SFOx rats maintained normal arterial blood pressure when chronically fed low- or high-salt diets. These results do not support the hypothesis that the SFO is necessary to maintain normotension under conditions of chronic changes in salt intake. On the other hand, we found less sodium retention in SFOx rats fed a high-salt diet than in SHAM rats, despite the ability of SFOx rats to maintain normal arterial pressure. These results suggest that the SFO may be important in the long-term neurogenic control of renal sodium excretion and homeostasis during chronic changes in dietary salt intake.

**GRANTS**

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